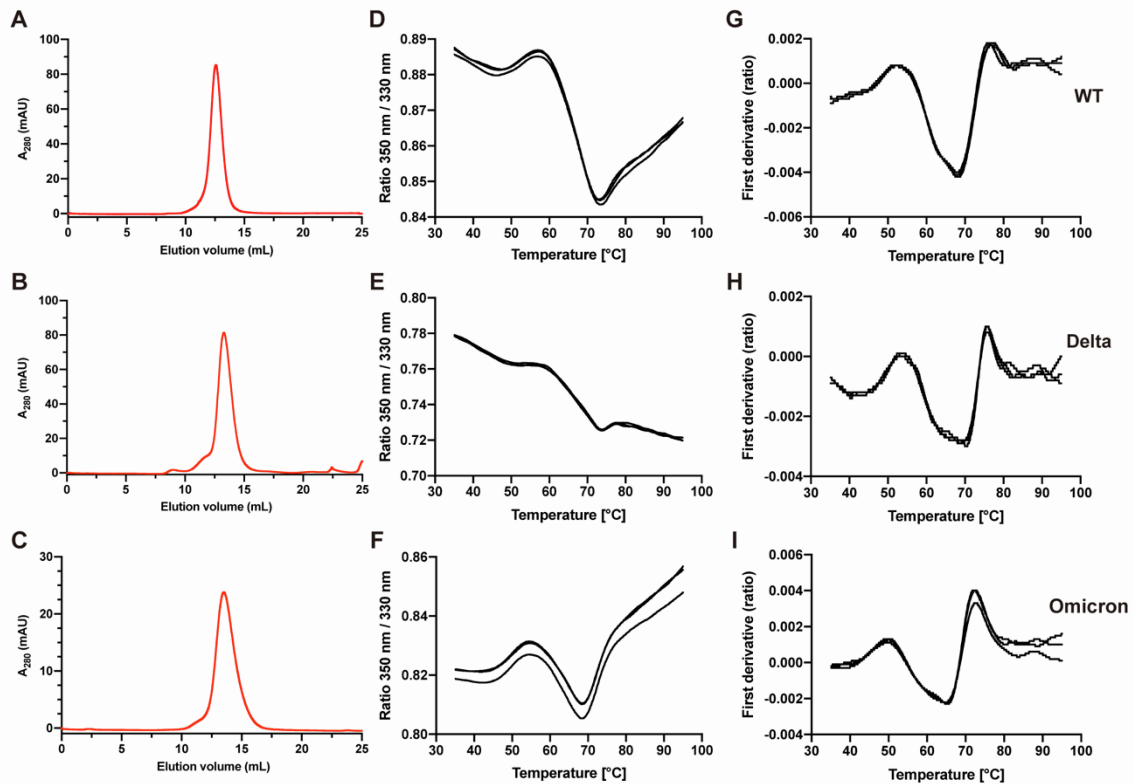


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Supplemental information

**Structures of Omicron spike complexes
and implications for neutralizing
antibody development**

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| Capillary label | Ti#1(°C) | Ti#2(°C) | Ti#3(°C) |
|-----------------|----------|----------|----------|
| S-WT | 52.6 | 68 | 76.43 |
| S-Delta | 53.8 | 71.6 | 75.7 |
| S-Omicron | 49.8 | 64.6 | 72.4 |

Ti, inflection temperature value determined in PBS buffer.

Figure S1 | Thermal denaturation analysis of the Spike variants, Related to Figure 1.

(A-C) SEC profile of the Spike proteins on a Superose 6 increase 10/300 column.

(D-F) The unfolding profile of Spike proteins (ratio between fluorescence at 350 nm and 330 nm) in real-time as the temperature is increased from 35°C to 95°C.

(G-I) The first derivative of the ratio between fluorescence at 350 nm and 330 nm. Maxima and minima indicate inflection temperatures, Ti.

(J) Summary of inflection temperatures for the Spike proteins.

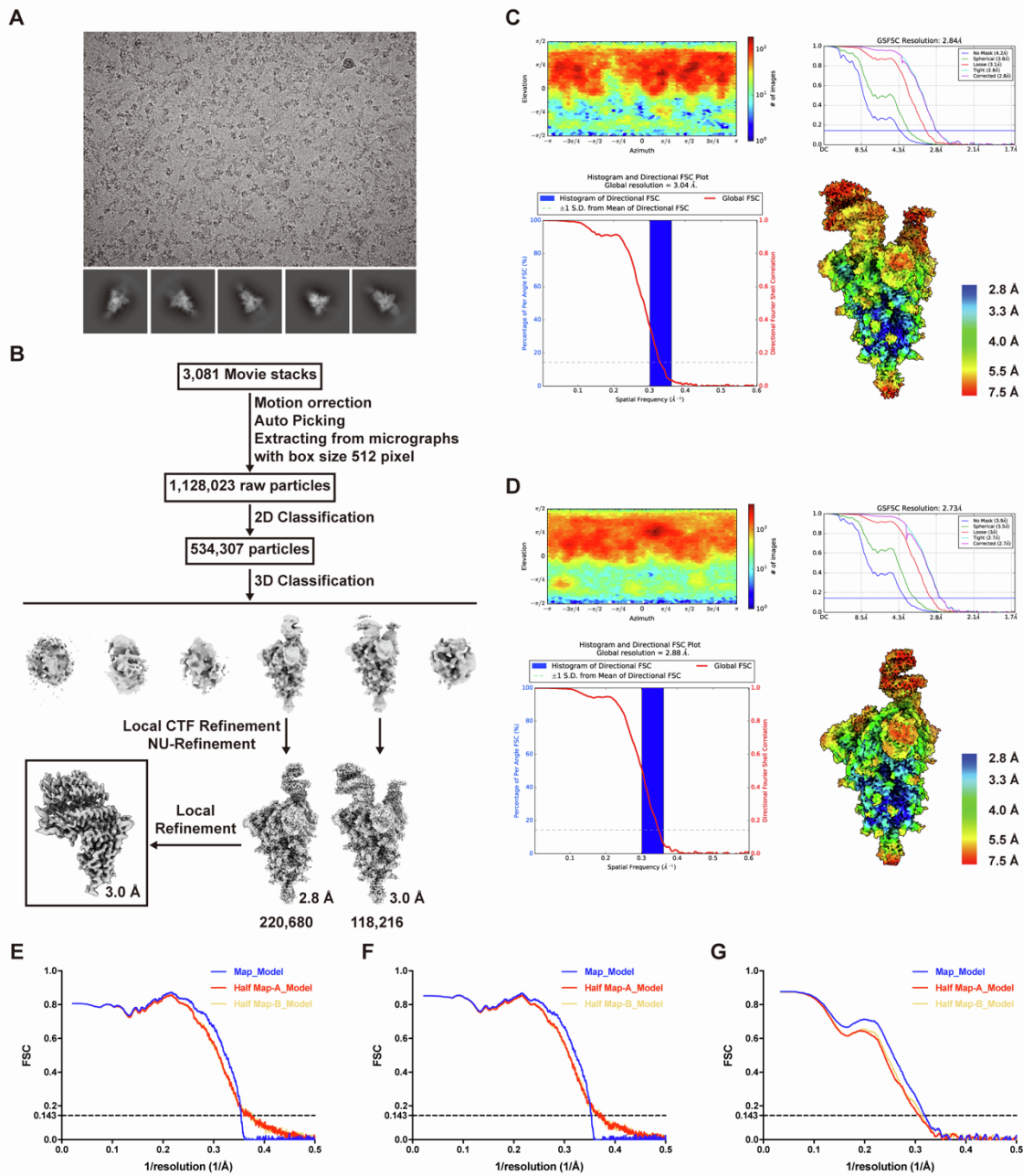


Figure S2 | Cryo-EM data processing for the Omicron Spike protein and human ACE2 complex, Related to Figure 1.

(A) Representative cryo-EM micrograph and 2D classes.

(B) Workflow for the Omicron Spike-ACE2 3D Reconstructions.

(C and D) The viewing direction distribution plot, gold-standard FSC curves with the 0.143 cutoff indicated by a horizontal blue line, Global FSC and Histogram, and cryo-EM maps colored by local resolution for the double (C) and single (D)-bound hACE2 classes, respectively.

(E-G) Map-to-model FSC curves of the double (E), single (F)-bound hACE2 classes as well as the local-refined dataset (G), respectively.

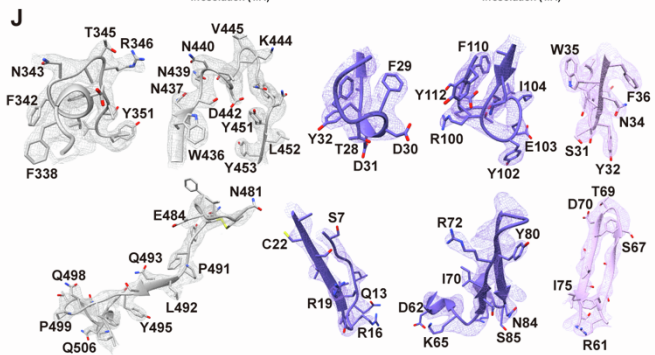
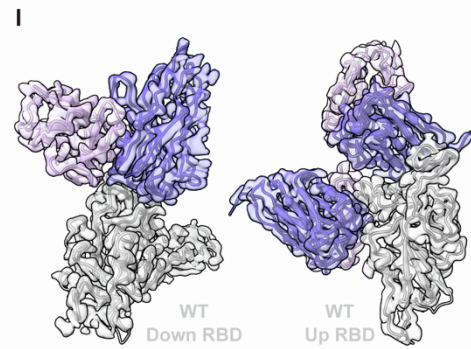
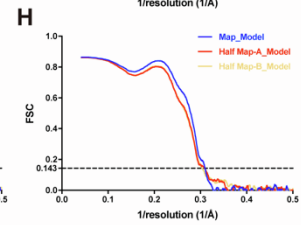
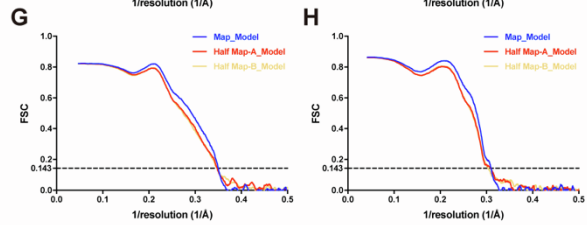
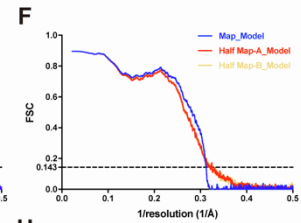
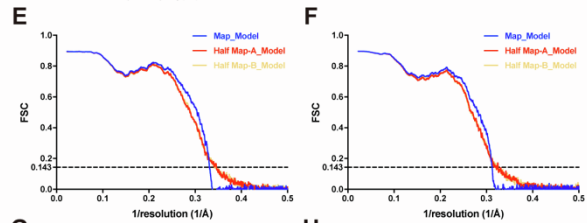
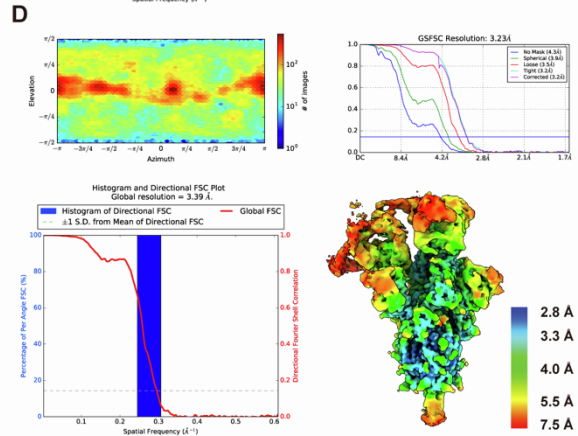
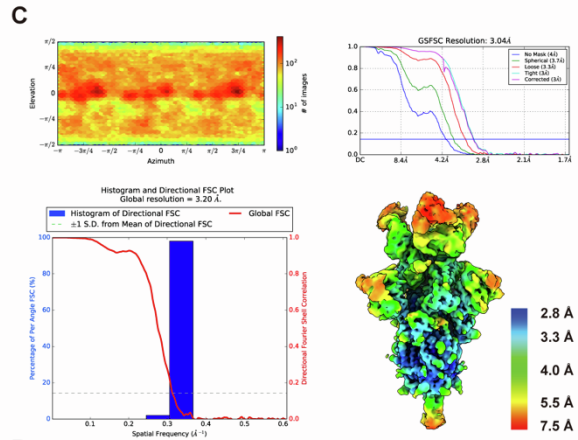
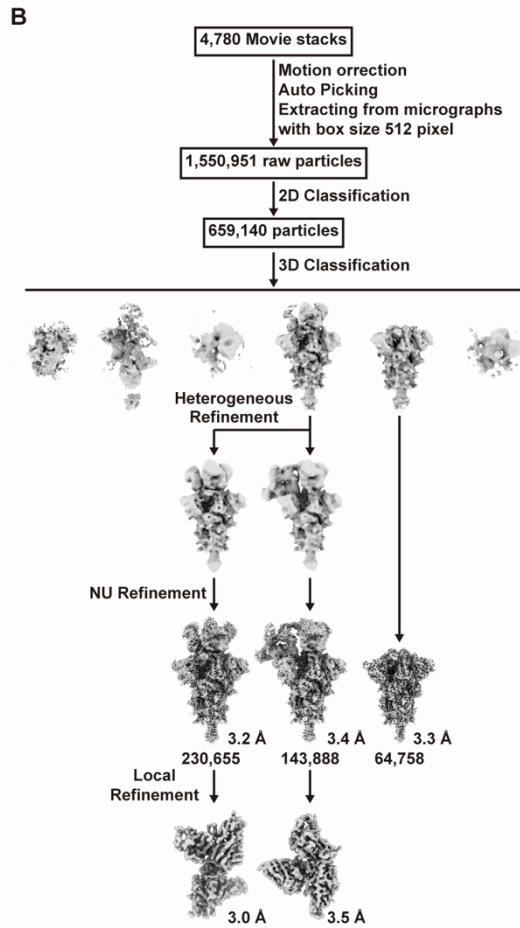
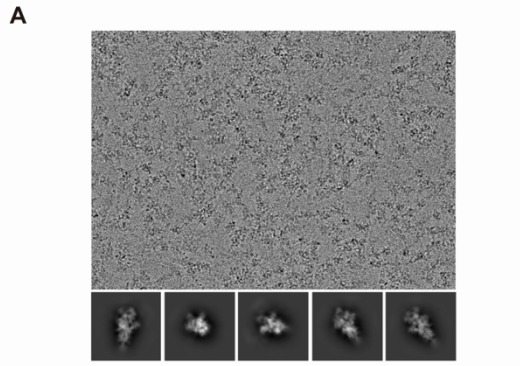


Figure S3 | Cryo-EM data processing for the SARS-CoV-2 WT Spike protein in complex with 510A5 Fab, Related to Figure 3.

(A) Representative cryo-EM micrograph and 2D classes.

(B) Workflow for the WT Spike-510A5 Fab 3D Reconstructions.

(C and D) The viewing direction distribution plot, gold-standard FSC curves with the 0.143 cutoff indicated by a horizontal blue line, Global FSC and Histogram, and cryo-EM maps colored by local resolution for the WT class I (C) and WT class II (D) datasets, respectively.

(E-H) Map-to-model FSC curves of the whole or locally refined reconstructions of the WT class I (E or G) and WT class II (F or H) datasets, respectively.

(I) Model-map fitting of the local-refined WT RBD-510A5 structures with RBDs in the down (left) and up (right) conformation, respectively.

(J) Sharpened cryoEM density maps (WT RBD and 510A5 Fab interaction regions) are rendered as mesh with the corresponding models shown as sticks.

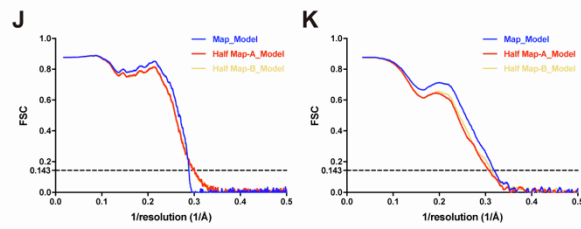
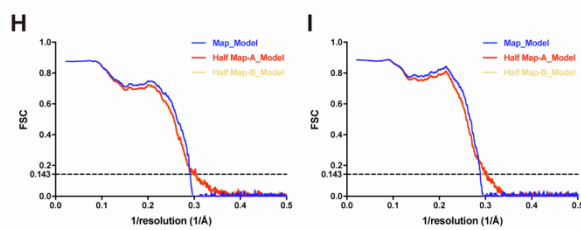
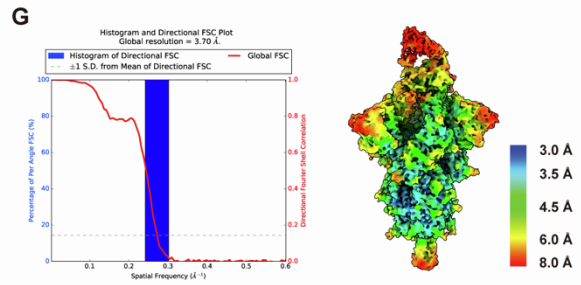
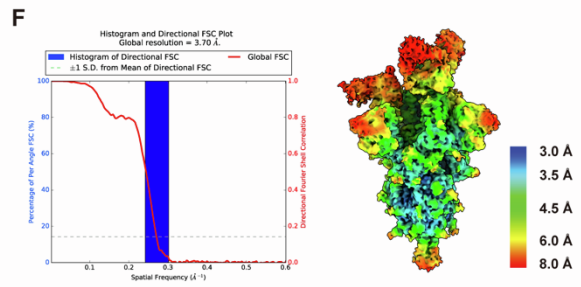
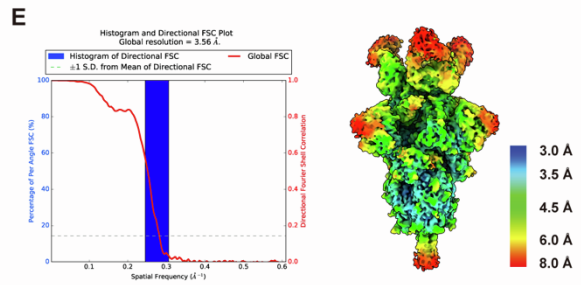
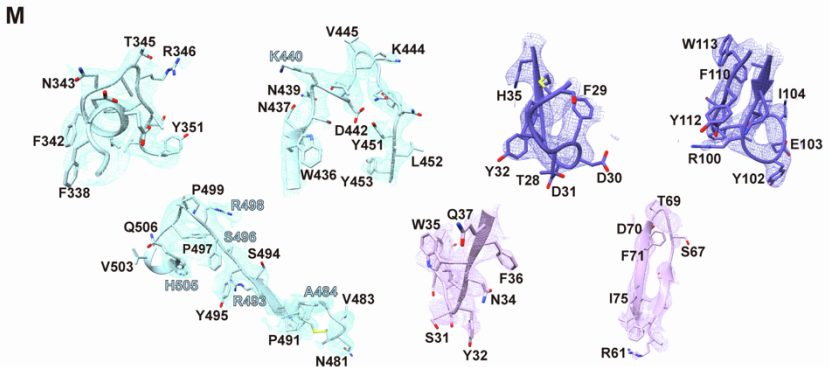
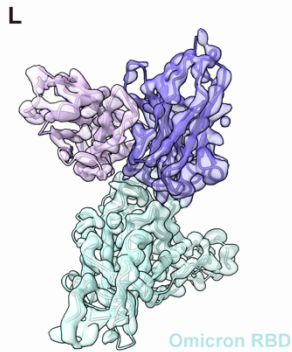
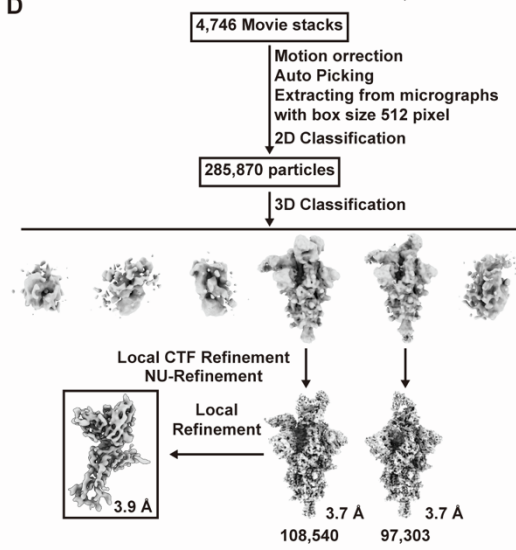
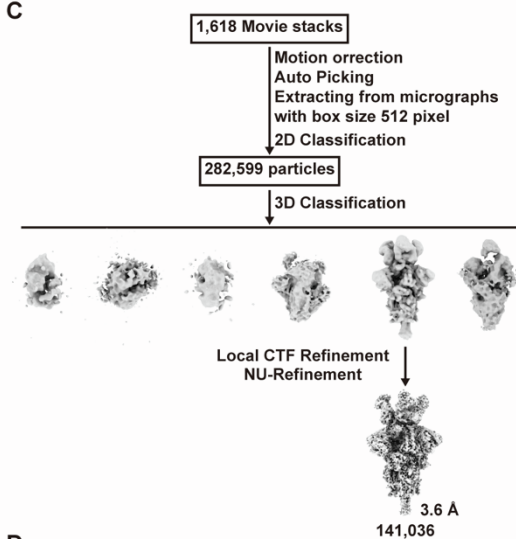
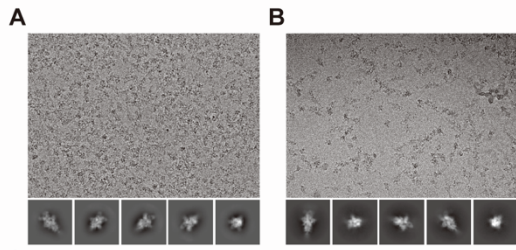


Figure S4 | Cryo-EM data processing for the SARS-CoV-2 Delta and Omicron Spike protein in complex with 510A5 Fab, Related to Figure 3.

(A and B) Representative cryo-EM micrograph and 2D classes for the Delta (A) or Omicron (B) Spike dataset, respectively.

(C and D) Workflow for the Delta Spike-510A5 Fab (C) and the Omicron Spike-510A5 Fab (D) 3D Reconstructions.

(E-G) The viewing direction distribution plot, gold-standard FSC curves with the 0.143 cutoff indicated by a horizontal blue line, Global FSC and Histogram, and cryo-EM maps colored by local resolution for the Delta (E), Omicron class I (F) and Omicron class II (G) datasets, respectively.

(H-K) Map-to-model FSC curves of the whole reconstructions of the Delta (H), Omicron class I (I) and WT class II (J) datasets, respectively, as well as the locally refined Omicron RBD-510A5 interface reconstructions (K).

(L) Model-map fitting of the local-refined Omicron RBD-510A5 structures.

(M) Sharpened cryoEM density maps (Omicron RBD and 510A5 Fab interaction regions) are rendered as mesh with the corresponding models shown as sticks.

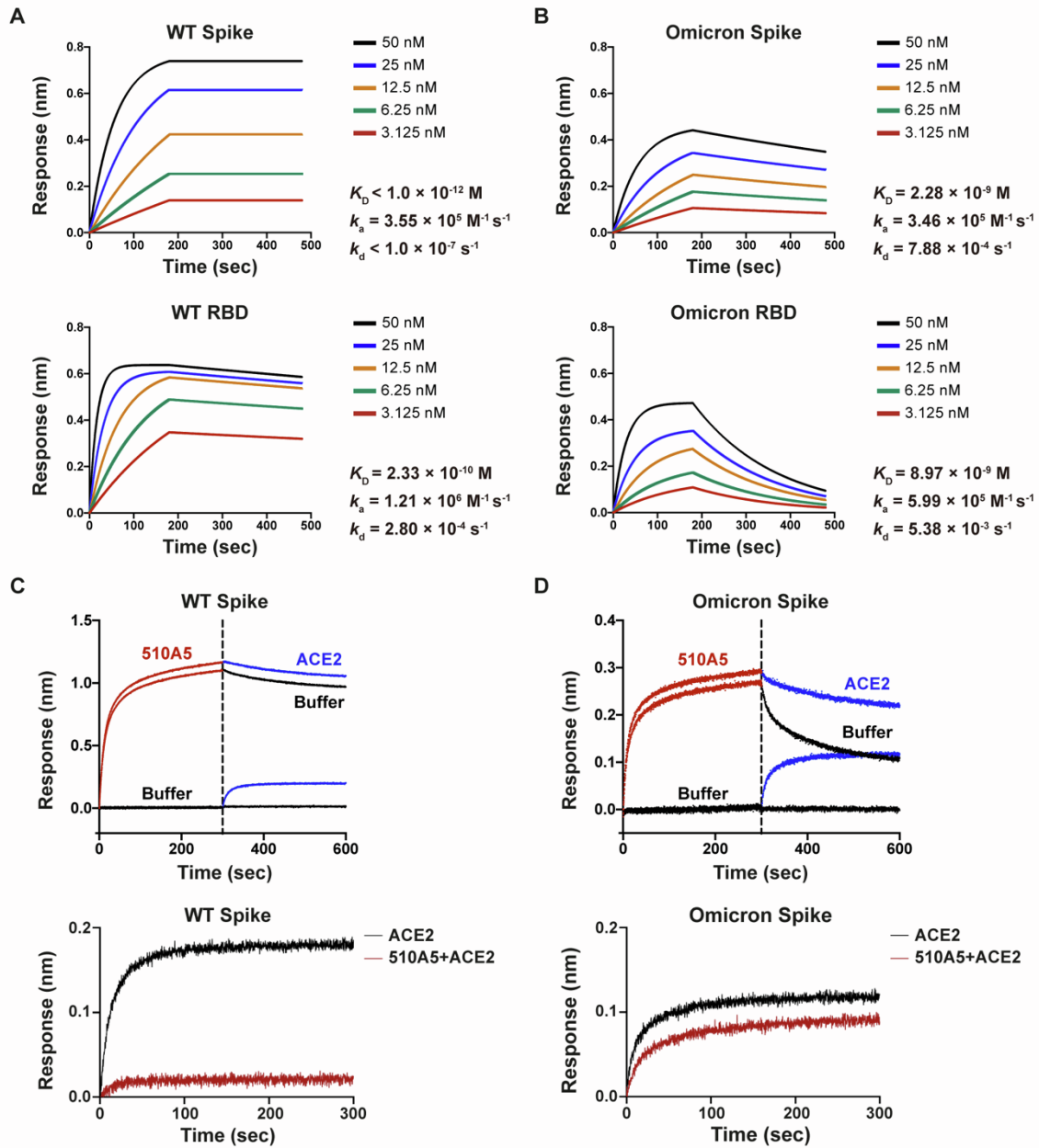


Figure S5 | Biolayer interferometry assays, Related to Figure 4.

(A and B) The binding kinetics of 510A5 to WT (A) or Omicron (B) Spike and RBD.

(C and D) The ability of 510A5 competing with ACE2 for binding to WT (C) or Omicron (D) Spike. The competition capacity of 510A5 was indicated by the level of reduction in the response unit of ACE2 compared with or without prior antibody incubation.

Table S1 | Cryo-EM data collection, models refinement and validation statistics of the Omicron Spike-ACE2 datasets, Related to Figure 1.

| | 2-ACE2 Bound | 1-ACE2 Bound | ACE2-RBD Local |
|--|--------------|--------------|----------------|
| Data collection and processing | | | |
| Voltage (kV) | 300 | 300 | 300 |
| Detector | K3 | K3 | K3 |
| Pixel size (Å) | 0.832 | 0.832 | 0.832 |
| Electron dose (e ⁻ / Å ²) | 60 | 60 | 60 |
| Defocus range | -1.2 to -2.8 | -1.2 to -2.8 | -1.2 to -2.8 |
| Final particles | 106,020 | 220,680 | 220,680 |
| Final resolution (Å) | 2.84 | 2.77 | 2.96 |
| Model refinement | | | |
| Map-model CC (mask) | 0.82 | 0.84 | 0.71 |
| Initial model used | 7T9K | 7T9K | 7T9L |
| RMSD | | | |
| Bond lengths (Å) | 0.004 | 0.003 | 0.004 |
| Bond angles (°) | 0.548 | 0.560 | 0.765 |
| Molprobability score | 1.59 | 1.53 | 1.73 |
| Clash score | 8.22 | 7.93 | 11.30 |
| Rotamer outliers (%) | 0.00 | 0.00 | 0.00 |
| Cβ outliers (%) | 0.00 | 0.00 | 0.00 |
| CaBLAM outliers (%) | 1.75 | 1.76 | 0.89 |
| Ramachandran statistics | | | |
| Favored (%) | 97.26 | 97.52 | 97.10 |
| Allowed (%) | 2.74 | 2.48 | 2.90 |
| Outliers (%) | 0.00 | 0.00 | 0.00 |

